JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1990, p. 1842–1846
0095-1137/90/081842-05$02.00/0

Vol. 28, No. 8

Transient Intestinal Colonization by Multiple Phenotypes of *Aeromonas* Species during the First Week of Life

G. PAZZAGLIA,[1,2]* J. R. ESCALANTE,[1,3] R. B. SACK,[2] C. ROCCA,[3] AND V. BENAVIDES[3]

U. S. Naval Medical Research Institute Detachment (Lima, Peru), A.P.O. Miami, Florida 34031[1]; Department of Immunology and Infectious Diseases and Department of International Health, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland 21205[2]; and Centro Medico Naval, Callao, Peru[3]

Received 20 February 1990/Accepted 21 May 1990

The intestinal colonization rate of *Aeromonas* spp. was determined for 52 cesarean-born Peruvian neonates. Rectal swabs were obtained daily from newborns during their postdelivery hospitalization ($\bar{x}$ = 5.5 days), and the gross appearances of their feces (blind determinations) were recorded. *Aeromonas* spp. were recovered from rectal swabs of 12 of 52 (23.1%) infants during their first week of life; the isolates were obtained from 5 of 9 (55.6%) infants with at least one stool with a watery consistency and from 7 of 43 (16.3%) neonates with no watery stools (P = 0.022). None of the infected infants became clinically ill. No other commonly recognized enteropathogens were detected in watery stools. An environmental survey indicated that hospital water was the probable source of infection. These and other data indicated that *Aeromonas* colonization occurs transiently at a very early age in Peruvian neonates and that in some instances, initial infection may be followed several days later by one or more watery stools of normal volume.

The role of *Aeromonas* spp. as significant diarrheal disease agents is controversial. These organisms have been epidemiologically associated with acute diarrhea in some controlled studies (1, 3) but not in others (4, 9), and surveys have revealed isolation frequencies ranging from 0.2 (6) to 22.9% (B. A. Kay, J. R. Harris, J. D. Clemens, D. A. Sack, A. Huq, R. Rahman, and A. K. J. Hasan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C282, p. 375) in diarrheic individuals. Oral challenges with *Aeromonas* spp. failed to cause significant diarrhea in adult volunteers (8), although *Aeromonas* strains have been shown to produce numerous potential virulence factors (5).

Recently, a hospital-based case control study of Peruvian children showed that the prevalences of *Aeromonas* infection among hospitalized diarrheic and healthy Peruvian children were 52.4 and 8.7%, respectively, and that healthy as well as diarrheic children were frequently infected with *Aeromonas* spp. before 2 months of age (R. B. Sack, G. Pazzaglia, E. Salazar, A. Yi, and E. Chea, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, C252, p. 435). These early colonizations by *Aeromonas* spp. in Peruvian children were confirmed by a separate community-based prospective study of newborns in which *Aeromonas* spp. were sometimes isolated from routine stool samples from children less than 1 month of age (G. Pazzaglia, R. B. Sack, R. Leon-Barua, A. Yi, J. Kohatsu, J. Benamu, C. E. Guerrero, and J. Palomino, Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1009, 1989). The clinical importance of these early infections or colonizations by *Aeromonas* spp. is unknown. The possibility exists that the first exposure to this organism normally results in a transient colonization that rarely causes any ill effects in the host.

By studying a cohort of normal newborns delivered by cesarean section, our primary objective was to determine how early in life intestinal colonization with *Aeromonas* spp. occurs by estimating the incidence rate of early neonatal fecal isolations of *Aeromonas* spp. If early infections by *Aeromonas* spp. were detected in hospitalized newborns, the secondary objectives were to document any associated clinical signs or symptoms and to identify the probable source of infection.

(These data were presented in part at a poster session at the 89th Annual Meeting of the American Society for Microbiology, New Orleans, La., 14 to 18 May 1989 [J. R. Escalante, G. Pazzaglia, C. Rocca, C. Benavides, and R. L. Buck, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, C251, p. 435].)

MATERIALS AND METHODS

Study design. This study was conducted during July through December, which is midwinter through midsummer in Lima, Peru. Lima is located in a coastal desert, but the climate is moderated by the offshore Humbolt Current (mean monthly temperatures vary from 15 to 22°C). The peak diarrhea season occurs in the summer (November through February).

A sequentially enrolled cohort of newborns delivered by cesarean section provided daily fecal specimens for enteropathogen testing. Cesarean section-delivered infants were selected as study subjects because they provided a unique opportunity to detect first exposure and early *Aeromonas* infection and/or colonization of infants in a clean, controlled environment. Rectal swabs were obtained from infants daily until their release and then again on the 15- and 30-day follow-up visits. At the time each specimen was collected, the investigator (always the same one) made an empirical determination of stool consistency and appearance. The observer was blind to previous data and laboratory results. Daily stool characteristics and other information were later compared with bacteriological outcomes.

Mothers and infants were monitored for a maximum of 45 days postdelivery, including the initial period of hospitalization for the mother (usually 5 to 7 days), the 15-day surgical follow-up visit, and the 30-day immunization follow-up visit.

Entry criteria. After a brief preliminary interview and their informed consent, pregnant mothers hospitalized for cesarean section delivery were enrolled for study. To obtain demographic and nutritional information, mothers were in-

* Corresponding author.

terviewed before surgery and during each follow-up visit. Study infants were born after no less than 35 weeks in utero. Enrollment was cancelled if the mother delivered a child considered unhealthy by the attending physician. The U. S. Navy's guidelines for the use of human subjects were followed in the conduct of this research.

***Aeromonas* isolations.** All fecal specimens for *Aeromonas* testing were obtained by using sterile rectal swabs. At the time of collection, swabs were placed directly into 10 ml of alkaline peptone water for enrichment. After overnight incubation at 20°C, alkaline peptone water enrichment broth samples (0.1 ml) were subcultured onto 5% sheep blood agar plates containing 10 μg of ampicillin per ml and incubated for 18 to 24 h at 28°C. Suspect colonies were tested for the presence of oxidase and then further characterized by the API 20E system (Analytab Products, Plainview, N.Y.). Supplemental tests (10) were performed to confirm that isolates belonged to the genus *Aeromonas* and to determine metabolic phenotypes according to the taxonomic scheme of Popoff and Veron (11). All *Aeromonas* isolates grew in nutrient broth without NaCl, did not grow in 6% NaCl nutrient broth, and were resistant to the vibriostatic agent O-129.

Other bacteriological testing. Additional rectal swabs were obtained from neonates who passed stools considered to be more watery than normal (by the blind observer). These were tested for the presence of enteropathogenic and enterotoxigenic *Escherichia coli*, *Vibrio* spp., *Campylobacter* spp., *Plesiomonas* spp., *Shigella* spp., and *Salmonella* spp. by using standard bacteriological methods (7). For rotavirus testing, a rectal swab was placed in phosphate-buffered saline and frozen (−85°C) until tested by enzyme-linked immunosorbent assay (Rotaclone; Cambridge BioScience, Worcester, Mass.). For parasite testing, a portion of stool was placed in Merthiolate-iodine-Formalin solution and assayed by concentration and direct microscopic examination.

Environmental survey. During the first and last months of the study, additional specimens from the hospital environment, mothers, and hospital personnel were cultured to identify possible sources of *Aeromonas* infection. Specimens from the unwashed hands of neonatal unit personnel were obtained during the course of their normal duties without prior notice. Surface specimens were collected by wiping with sterile gauze or cotton-tipped swabs soaked with sterile 0.85% saline or phosphate-buffered saline and then placing the swabs directly into alkaline peptone water. Water specimens were collected midstream into a sterile container and filtered (pore size, 0.45 μm), and the filter was then placed in 10 ml of alkaline peptone water.

Nutrition and weight measurements. A detailed daily record of the feeding history of each neonate was recorded for the initial period of hospitalization, including the times all meals were taken, whether formula or breast feeding was used, and formula volumes. Weights of the neonates were measured and recorded for each day during the mothers' hospitalization and on each follow-up visit.

RESULTS

During the 6-month period, 52 neonates were enrolled for study. Neonates were cultured daily during the postpartum hospitalization period ($\bar{x}$ = 5.5 days) and then again during the 15- and 30-day follow-up visits. In total, there were 342 rectal swabs cultured, averaging 6.6 specimens per child. Compliance of the mothers was high, with 80.8% of mothers returning for at least one follow-up visit.

TABLE 1. *Aeromonas* fecal isolations, by age, from a cohort of 52 Peruvian neonates

No. of days postpartum	No. (%) of *Aeromonas*-positive neonates	
	Daily[a]	Cumulative[b]
1	2/52 (3.9)	2/52 (3.9)
2	7/52 (13.5)	8/52 (15.4)
3	2/49 (4.1)	9/52 (17.3)
4	4/46 (8.7)	10/52 (19.2)
5	4/44 (9.1)	11/52 (21.1)
6	3/24 (12.5)	12/52 (23.1)
7–9	0/20 (0.0)	12/52 (23.1)
15	1/31 (3.2)	13/52 (25.0)
30	2/24 (8.3)	15/52 (28.8)

[a] The denominator is the number of neonates tested on each day postpartum. This group of neonates decreased in number through day 9 because the cohort was reduced as the mother-infant pairs were released from the hospital. Denominators on days 15 and 30 are the numbers of mother-infant pairs who returned for follow-up visits after their discharge.

[b] Denominators represent the total cohort. As neonates became positive for *Aeromonas* spp., they were accrued into the numerator on the first day of isolation. (As an example, by day 3 postpartum, 9 of 52 neonates had produced at least one positive stool specimen, including the 2 positive specimens among the 49 neonates tested on day 3.)

Aeromonas spp. were isolated from 12 of 52 (23.1%) neonates by 6 days postpartum and from 15 of 52 (28.8%) infants by 1 month of age (Table 1). Of the three infants who had *Aeromonas* spp. isolated from their stools on the 15- or 30-day follow-up visit, only one had a previous culture-positive stool. Of the 52 neonates, 6 had multiple *Aeromonas* isolations, and 4 of these had *Aeromonas* spp. isolated from their stools on at least 2 consecutive days. However, of the 12 initial isolations during the first 6 days postpartum, only 3 were followed by an isolation on the next day. Of the 12 neonates who were culture positive during the first week of life, only one was culture positive on the 15- or 30-day follow-up visit.

Eight infants had a single day of watery stools, and one had two days of watery stools which were three days apart. During the first week of life, infants with at least one watery stool were more likely to have *Aeromonas* spp. recovered from rectal swabs than were infants without a watery stool (P = 0.022; Table 2). However, only 2 of the 15 initial

TABLE 2. Watery stools and their association with *Aeromonas* isolations among a cohort of 52 Peruvian neonates cultured daily from birth[a]

No. of days postpartum	No. of watery stools observed				P^b
	At least one		None		
	No. of neonates tested	No. (%) with *Aeromonas* spp.	No. of neonates tested	No. (%) with *Aeromonas* spp.	
1	1	0 (0.0)	51	2 (3.9)	1.000
2	5	2 (40.0)	47	6 (12.8)	0.164
3	7	3 (42.9)	45	6 (13.3)	0.082
4	8	4 (50.0)	44	6 (13.6)	0.035
5	9	5 (55.6)	43	6 (14.0)	0.014
6	9	5 (55.6)	43	7 (16.3)	0.022
7	9	5 (55.6)	43	7 (16.3)	0.022

[a] Not all neonates were monitored the full 7 days.

[b] Significance of comparison between group with at least one watery stool and group with no watery stools as determined by Fisher's exact test.

Aeromonas isolations were made from watery stools. Among the three infants from whom *Aeromonas* spp. were recovered on 2 consecutive days during the initial infection, only one of the six isolates was from a watery stool.

There were no significant differences in frequency of isolation when study infants were grouped according to formula- and breast-feeding patterns or weight at birth, and there was no correlation between *Aeromonas* isolation and percent weight gain. No seasonal trends were detected for frequencies of *Aeromonas* isolation or watery stools. None of the neonates producing watery stools showed any other sign or symptom indicative of enteric illness or other disease during their hospital stay. No other known or suspected enteropathogens were detected.

Of the 21 *Aeromonas* isolates, 7 did not survive long enough to be phenotyped. Among the six surviving fecal isolates from neonates who had at least one watery stool, four were of the *A. hydrophila* phenotype, one was *A. caviae*, and one was *A. sobria* (phenotyping was based on the ability to utilize salicin, arabinose, or arginine; esculin hydrolysis; production of H_2S from cysteine; and production of acetoin from glucose [10]). From normal children, four of the eight surviving isolates were *A. caviae*, two were *A. hydrophila*, and two were *A. sobria*.

Of the three neonates who had multiple isolates of *Aeromonas* spp. which were phenotyped, only one yielded the same phenotype on each occasion (*A. hydrophila* from a neonate who had watery stools; isolated on days 3 and 5 postpartum). *A. hydrophila* and *A. caviae* were isolated on days 3 and 6 postpartum, respectively, from another infant who had watery stools. One normal child had *A. hydrophila* isolated from a stool on day 2 and *A. caviae* isolated on days 3 and 4 postpartum.

Aeromonas spp. were isolated from unsterilized hospital water samples, prepared formulas, and bath basins but not from the mothers, hospital personnel, or cultured surfaces (Table 3). Isolates from sources other than neonates were not phenotyped.

TABLE 3. Bacteriological survey results of *Aeromonas* isolations from study mothers, hospital personnel, and the hospital environment

Type or origin of specimen	Type or quantity of specimen	*Aeromonas* isolations/no. of specimens (%)
Water		
Bottle-washing area	3 ml	2/4 (50.0)
Neonate baths	100 ml	2/4 (50.0)
Sterilized water for formula	100 ml	0/4 (0.0)
Infant formula		
Dry (unprepared)	3 g	0/3 (0.0)
Prepared (unused)	3 ml	1/4 (25.0)
Prepared (after feeding)	3 ml	0/3 (0.0)
Feeding bottle (sanitized)	Swab	0/3 (0.0)
Mother's nipple	Swab	0/22 (0.0)
Mother of *Aeromonas*-positive neonate		
Rectum	Swab	0/2 (0.0)
Hands	Swab	0/2 (0.0)
Neonatal unit personnel (hands)	Swab	0/8 (0.0)
Surfaces		
Basin (mothers's bath)	Swab	2/4 (50.0)
Floor (neonatal unit)	Swab	0/4 (0.0)
Counter tops, incubators, and carts	Swab	0/7 (0.0)

DISCUSSION

The study was terminated after the enrollment of 52 neonates because the specific objectives were met and because we desired to minimize the number of neonates studied, despite the limited risks to the child. The results confirmed that *Aeromonas* infection is endemic in Lima, and established that Peruvian children are frequently colonized with these organisms during the first week of life. The outcome was especially striking in that these early infections occurred in the sanitized environment of a neonatal unit in a private hospital. The colonizations, when they occurred, appeared to be transient and not associated with an illness more severe than a single day of watery stools of normal volume.

If it were feasible to perform a similar study with a cohort of infants encountering their first *Aeromonas* exposure at the time when maternal antibody levels were declining, the resultant colonizations could, conceivably, be accompanied by clinical signs or symptoms. However, performing such a study, while ensuring that the observed colonizations were a result of the first exposure, would require daily fecal specimens for at least the first 3 months of life, which may well be an unattainable objective. Additionally, considering that only a limited number of Peruvian infants will not have been exposed to or colonized with *Aeromonas* spp. by 3 months of age, the study would require an excessively large cohort.

The findings of the hospital environmental survey provided evidence that water, and not fomites or direct contact with mothers or hospital personnel, was the principal source of the *Aeromonas* infections. *Aeromonas* spp. were not isolated from water used for formula preparation but were only isolated from water used for bathing and bottle washing and from one bottle of prepared formula not yet used. Because the dry formula, sanitized formula bottles, and sterile water for formula preparation that were cultured were negative for *Aeromonas* spp., it is probable that the single bottle of *Aeromonas*-positive prepared formula was contaminated after or during preparation.

During the survey, we cultured samples from the breasts of 22 of the mothers, hypothesizing that if mother-to-neonate transmission were occurring, breast-feeding from a contaminated nipple was the most likely source of infection, even if the organism had originated from the mother's feces. The results indicated this was not a significant mode of transmission. If the mother's feces were the ultimate source, the other major mechanism of transmission to consider was direct fecal transmission via the mother's fingers, which we believed was a less likely event. Only two specimens from mothers' hands and two from mothers' rectums were obtained. Although all four were culture negative, all were from mothers of *Aeromonas*-positive infants. Obtaining additional rectal swabs from mothers seemed inappropriate, primarily because mothers recovering from surgery were not receptive to the procedure. Fecal cultures from hospital personnel would have been helpful in our transmission investigation, but it would have been difficult to justify asking hospital personnel to submit to rectal swabbing or to contribute daily stool specimens for a study of healthy infants. The results from our limited environmental survey suggested that *Aeromonas* transmission in the hospital neo-

natal unit occurred either when infants were fed previously sterile formula which had been contaminated during preparation or from ingestion of contaminated water when the infants were being bathed.

The transient nature of most initial infections with *Aeromonas* spp. was evidenced by several study results. *Aeromonas* spp. were infrequently isolated from rectal swabs on consecutive days (3 of 12 [25.0%] initial isolates were followed by a positive culture on the next day; of 15 specimens from neonates who were culture positive on the previous day, *Aeromonas* spp. were isolated from 5 [33.3%]). Furthermore, multiple isolations from the same neonate were usually of different phenotypes (of the three neonates who had multiple surviving isolates, two had isolations of different phenotypes). Finally, most of the neonates who were culture positive during the first week of life were culture negative on the 15- and 30-day follow-up visits (11 of 12 [91.7%]).

We are confident that the differences in isolation frequencies between normal and watery stool groups were real because the study groups were defined on the basis of stool characteristic observations which were performed by a single investigator (J.R.E.) who was blind to laboratory results. The association of *Aeromonas* infection with an increased likelihood of a neonate having at least one watery stool was also demonstrated when results were analyzed according to individual stools instead of individual neonates: *Aeromonas* spp. were isolated from 4 (40.0%) of the 10 watery stool samples cultured, compared with 18 (5.4%) of 332 normal stool samples. This comparison, although not statistically valid because the stools were not sampled independently, further supports the notion that initial infections may be associated with a single day of "loose" stools.

Although the lack of objective stool classification criteria created a potential source of classification error (i.e., watery versus nonwatery stools), it could not have introduced bias or affected the validity of the observed associations with stool consistency because of the blind observer. There was no reasonable chance that the stool classifications could have influenced the outcome of the laboratory testing; other than delivering the specimens, the observer had no laboratory responsibilities. Because the observer could not have introduced bias into laboratory results, substantive misclassification error on his part would have tended to increase the likelihood of failure to detect a real association rather than the likelihood of erroneous detection of a nonexistent association.

Whether the watery stools observed in this study can be considered diarrheic is a question of arbitrary definition. We chose not to call them diarrheic because the episodes were of only 1 day's duration, not excessive in volume, and not accompanied by other signs or symptoms. The difficulty in applying objective, well-defined criteria to describe the gross appearances of stools for children of this age group is obvious to those who have observed the variability that occurs in fecal characteristics for an individual infant during the first week of life. In addition, significant differences exist between infants, and these cannot always be attributed to dietary influence. Meconium stools, which are usually passed during the first 24 h, were not a significant factor in the current study because only one infant had what was considered to be a watery stool during the first 48 h of life.

If one accepts the premise that the observations of watery stools in the study neonates were clinically relevant, the isolation of *A. hydrophila* or *A. sobria* from 4 of 9 (44.4%) neonates with watery stools, compared with 4 of 43 (9.3%) infants with normal stools ($P = 0.023$), may have been significant in regard to the association of certain *Aeromonas* biotypes with human diarrhea. This contrasted with *A. caviae*, which was isolated from 1 of 9 (11.1%) infants with watery stools, compared with 3 of 43 (7.0%) normal infants ($P = 0.544$). From a different perspective, a higher proportion of isolates from neonates with normal stools were *A. caviae* (four of eight [50.0%] *Aeromonas* isolates phenotyped), compared with neonates who had watery stools (one of six [16.7%] *Aeromonas* isolates phenotyped). *A. caviae* has been found to infrequently produce enterotoxins which may cause watery stools (2). Several investigators have suggested that *A. caviae* is not an enteric pathogen (5), which is in agreement with the aforementioned observations and other studies we have performed in Peru.

The ubiquity of *Aeromonas* spp. in poor suburbs of Lima has been shown in a recent community-based study in which the organism was isolated from 89% of household environments, including 38% of household potable water samples (Pazzaglia et al., 29th ICAAC). The vast majority of births in Peru occur in homes or in public hospitals, often in unsanitary conditions. Although cesarean sections are commonly performed in private Peruvian hospitals, which serve a relatively small and affluent population, they are infrequently performed in public hospitals. These factors suggest that our in-hospital results greatly underestimated the true frequency of early initial colonization of newborns in the general Peruvian population. Because Peru has no nationwide system of health care reporting, it is impossible to accurately extrapolate from our results to the general population.

In summary, the results of this study have demonstrated that most children in Peru do not develop clinical illness upon their first exposure to *Aeromonas* spp., regardless of the metabolic phenotype of the infecting strain. The clinical observations suggested that initial colonization may be followed by transient watery stools several days later. Considering the recently recognized diversity of the genus *Aeromonas*, it is unlikely that specific maternal antibody was responsible for the lack of disease association. It is more probable that only a minority of *Aeromonas* strains are overtly diarrheagenic, and that such *Aeromonas* isolates were not represented in the current study. Results also indicated that these early infections, most likely from a contaminated water source, did not result in substantial or long-term colonization. The infrequent recovery of *Aeromonas* strains (and specific phenotypes) on consecutive days suggested either that colonization was little more than the intestinal passage of actively multiplying bacteria or that the numbers of colonizing organisms shed in feces were frequently below the minimum levels of detection.

ACKNOWLEDGMENTS

This work was supported by the U.S. Naval Medical Research and Development Command, Bethesda, Md., Research Projects 62770A 3M162770.A870.AR-522 and 61102A 3M161102.BS13.AK-511, and the Surgeon General's Office of the Peruvian Navy.

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UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) NMRI 90-86	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Naval Medical Research 6b OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION Naval Medical Command
6c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055	7b. ADDRESS (City, State, and ZIP Code) Department of the Navy Washington, D.C. 20372-5120
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research and Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO
62787A	3M162787.A870	AR522	
61102A	3M161102.BS13	AK511	DA313956

11. TITLE (Include Security Classification)
Transient Intestinal Colonization by Multiple Phenotypes of Aeromonas Species during the First Week of Life

12. PERSONAL AUTHOR(S)
Pazzaglia G, Escalante JR, Sack RB, Rocca C, Benavides V.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
journal article	FROM _____ TO _____	1990	5

16. SUPPLEMENTARY NOTATION
Reprinted from: Journal of Clinical Microbiology, Aug. 1990, Vol.28 No.8 pp. 1842-1846

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Aeromonas, colonization, diarrhea, neonates, Peru

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Phyllis Blum, Information Services Division	22b. TELEPHONE (Include Area Code) 202-295-2188 22c. OFFICE SYMBOL ISD/ADMIN/NMRI

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
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